Laboratory Evaluation of Soybean Plant Introductions for Resistance to *Aphis glycines* (Hemiptera: Aphididae)\(^1\)

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**ABSTRACT** The soybean aphid (SA), *Aphis glycines* Matsumura, is a major pest of soybean in the north-central United States and south-central Canada. It is controlled primarily with insecticides, but the development of aphid-resistant soybean cultivars may provide an alternative management tactic. The viability of this management tactic depends on a diverse set of resistance sources in order to counter various resistance-breaking biotypes of SA, and the identification of new sources of resistance necessitates additional testing of soybean germplasm. The current study used no-choice tests to identify SA resistance in seven early maturing (maturity group I) soybean plant introductions (PIs) that had been advanced from free-choice screening trials. The tests showed PI 437353 and PI 612759 C had an intermediate level of resistance against avirulent SA, whereas PI 437282, PI 437658, PI 437733, PI 548417, and PI 548530 exhibited no significant resistance. Additional research is needed to determine if the source of resistance in PI 437353 and PI 612759 C is due to novel resistant genes, which would help diversify resistance to SA in soybean. Screening and follow-up tests of additional soybean germplasm is warranted in order to ensure the development of durable, SA-resistant cultivars.

**KEY WORDS** Host plant resistance, crop protection, invasive species, biotype 1

The soybean aphid (SA), *Aphis glycines* Matsumura (Hemiptera: Aphididae), has been a major pest of soybean in North America since 2000 and has the ability to inflict crop yield loss up to 40% (Ragsdale et al. 2007). Insecticides are the primary means of controlling this pest, contributing to a 130-fold increase in insecticide use in the north-central U.S. (Ragsdale et al. 2011). In addition, the freshwater ecotoxicological impact of insecticides used on soybean fields quintupled between 2002 and 2012 (Yang & Suh 2015). Thus, effective alternative management strategies are needed to lessen the environmental impact of SA control.

The development and use of soybean cultivars with genetic resistance to SA may help supplant the use of insecticides. Several soybean lines with resistance...
to SA have been identified (Hill et al. 2012, Hesler et al. 2013), and this has led to the development of aphid-resistant cultivars in the U.S. (Hodgson et al. 2012). However, their adoption by soybean growers has been limited by incomplete resistance due to at least three virulent SA biotypes that have overcome one or more aphid-resistance genes (Kim et al. 2008, Hill et al. 2010, Alt & Ryan-Mahmutagic 2013, Hesler et al. 2013).

Increasing the adoption and sustainability of aphid-resistant soybean cultivars may depend on the identification of multiple resistance genes that can be pyramided within a single soybean cultivar or used as individual resistance genes in multiple varieties (Smith 2005, Hesler et al. 2013). Using pyramided cultivars or employing multiple cultivars, each with a different source of resistance used in rotation or planted within the same field, will help decrease the chance of SA overcoming resistance genes. Additional SA resistance genes may be useful against known SA biotypes (Hesler et al. 2013, McCarville et al. 2014, Chandrasena et al. 2015, Ajayi-Oyetunde et al. 2016) and potentially against yet to be identified biotypes. However, the identification of diverse resistance sources depends on screening a large number of germplasm accessions (Boethel 1999, Smith 2005).

Hesler et al. (2017a, b) recently identified additional sources of SA resistance among 746 early-maturing (maturity group I; Pedersen & Licht 2014) soybean plant introductions (PIs) screened in a series of no-cage tests in which SA were free to colonize various test lines within environmental chambers. The resistant lines included PI 437282, PI 437353, PI 437658, PI 437733, and PI 612759 C. Hesler et al. (2017a) also identified two sources (PI 548417 and PI 548530) with strong resistance in some individual plants, but which categorized as susceptible overall.

Although no-cage choice tests efficiently screen large numbers of lines for resistance, their results may be unduly influenced by relatively high attractiveness of some susceptible lines rather than high unsuitability of lines scored as resistant (Harris 1980). Thus, putatively resistant sources identified in screening require further evaluation in no-choice cage tests that confine SA to individual lines and better simulate the limited choice of host lines that SA face under field conditions (Harris 1980, Davis 1985, Mensah et al. 2005, Hesler 2013). The objective of this study was to use no-choice tests to further evaluate a subset of putatively SA-resistant PI lines identified by Hesler et al. (2017a, b).

**Materials and Methods**

Putatively resistant PI lines were compared within a series of three no-choice tests that measured SA population growth over a 20-d period. Each test had five to seven soybean lines (Table 1), including a resistant (LD05R-16176) and a susceptible check (SD01-76R) (Hesler et al. 2013), and also `Davison’ (PI 662941), an additional known susceptible cultivar (Hesler et al. 2017a). Resistance in LD05R-16176 is attributed to its resistant parent, `Dowling,’ which contains the Rag1 resistance gene (Li et al. 2007). Test 1 included PI 437353 and PI 437658; test 2 included PI 437282, PI 437733, PI 548417, and PI548530; and test 3 included PI 548417 and PI 612759 C. Test 3 repeated PI 548417 and also included an additional known susceptible cultivar, PI 522322 B, because this test was initially an independent undergraduate research project. PIs were obtained from the USDA-ARS Soybean Germplasm Collection, National Soybean Research Center, Urbana,
Table 1. Overview of soybean lines used in three no-choice cage tests against soybean aphid.

<table>
<thead>
<tr>
<th>Test</th>
<th>Line</th>
<th>Description</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>All tests</td>
<td>LD05R-16137</td>
<td>resistant check</td>
<td>Hesler et al. 2013</td>
</tr>
<tr>
<td></td>
<td>SD01-76R</td>
<td>susceptible check</td>
<td>Hesler et al. 2013</td>
</tr>
<tr>
<td></td>
<td>Davison (PI 662941)</td>
<td>susceptible cultivar</td>
<td>Hesler et al. 2017a</td>
</tr>
<tr>
<td>Test 1</td>
<td>PI 437353</td>
<td>test line</td>
<td>Hesler et al. 2017a,b</td>
</tr>
<tr>
<td></td>
<td>PI 437658</td>
<td>test line</td>
<td>Hesler et al. 2017a,b</td>
</tr>
<tr>
<td>Test 2</td>
<td>PI 437282</td>
<td>test line</td>
<td>Hesler et al. 2017a,b</td>
</tr>
<tr>
<td></td>
<td>PI 437733</td>
<td>test line</td>
<td>Hesler et al. 2017a,b</td>
</tr>
<tr>
<td></td>
<td>PI 548417</td>
<td>test line</td>
<td>Hesler et al. 2017a,b</td>
</tr>
<tr>
<td></td>
<td>PI 548530</td>
<td>test line</td>
<td>Hesler et al. 2017a</td>
</tr>
<tr>
<td>Test 3</td>
<td>PI 522322 B</td>
<td>susceptible</td>
<td>Hesler et al. 2017a,b</td>
</tr>
<tr>
<td></td>
<td>PI 548417</td>
<td>test line</td>
<td>Hesler et al. 2017a</td>
</tr>
<tr>
<td></td>
<td>PI 612759 C</td>
<td>test line</td>
<td>Hesler et al. 2017a,b</td>
</tr>
</tbody>
</table>

The checks and Davison were obtained from South Dakota State University, Brookings, SD. Each test had six replicates and used a separate completely randomized design to arrange test entries.

Soybean aphids in the tests had been reared on ‘Brookings’ soybean (PI 667735; Jiang et al. 2014), and they constituted the same source of aphids used by Hesler et al. (2017a, b). Previous testing of the aphids determined that they were avirulent to several soybean lines with known resistance (i.e., Rag) genes and thus consistent with responses of SA biotype 1 (Hill et al. 2012, Hesler et al. 2017b).

Test plants were grown in plastic pots (6 cm top diameter, 4 cm bottom diameter, 5.7 cm height) containing a 2:1:1 mixture of Vienna soil (fine-loamy, mixed Calcid Hapludolls), vermiculite (Sta-Green, Infinity Lawn and Garden, Milan, IL) and sphagnum peat moss (Conrad Farfard Inc., Agawam, MA) (Hesler 2013). Three seeds of an individual PI line were placed ca. 3 cm deep into the soil mix, and the soil mix was saturated with water. Ten to 12 d later, seedlings were thinned to a single plant based on uniform growth among PI lines. The soil surface of each test pot was then covered with a ca. 2-cm layer of white sand (industrial quartz, Unimin Corporation, New Canaan, CT) that suppressed fungus gnats and stabilized cages that were used after aphid infestation (Hesler 2013, Hanson et al. 2016).

The tests were conducted using modified procedures from Hesler (2013) and run in environmental chambers (Conviron, CMP4030, Winnipeg, Canada) set with a 16:8 (L:D) h photoperiod and a 24:18°C (L:D) temperature range at the North Central Agricultural Research Laboratory, USDA-ARS, Brookings, SD. Tests commenced by transferring 10 adult SA from rearing plants onto each test plant (five SA per unifoliolate leaf, VC stage; Pedersen & Licht 2014) and immediately covering the plant with a clear, cylindrical, cellulose nitrate cage (10 cm diameter, 40 cm height). Twelve plants of each PI line were used per test; six were sampled 10 d after infestation by clipping the shoots at soil level, and the other six were clipped on Day 20. Test plants were put individually into sealable plastic bags, labeled, and placed in a freezer immediately after clipping. A few days
Table 2. Analyses of variance results for three no-choice tests in which soybean aphids were sampled on soybean test lines at 10 and 20 days after infestation.

<table>
<thead>
<tr>
<th>Test</th>
<th>Statistic</th>
<th>Test line</th>
<th>Sample day</th>
<th>Test line × sample day</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$F$</td>
<td>77.82</td>
<td>409.02</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td>d.f. (factor, error)</td>
<td>4, 50</td>
<td>1, 50</td>
<td>4, 50</td>
</tr>
<tr>
<td></td>
<td>$P$</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.0390</td>
</tr>
<tr>
<td>2</td>
<td>$F$</td>
<td>51.90</td>
<td>286.66</td>
<td>10.94</td>
</tr>
<tr>
<td></td>
<td>d.f. (factor, error)</td>
<td>6, 70</td>
<td>1, 70</td>
<td>6, 70</td>
</tr>
<tr>
<td></td>
<td>$P$</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>3</td>
<td>$F$</td>
<td>52.27</td>
<td>329.16</td>
<td>5.36</td>
</tr>
<tr>
<td></td>
<td>d.f. (factor, error)</td>
<td>5, 60</td>
<td>1, 60</td>
<td>5, 60</td>
</tr>
<tr>
<td></td>
<td>$P$</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.0004</td>
</tr>
</tbody>
</table>

d.f. = degrees of freedom.

later, the plants were removed and thawed, and the numbers of SA on them were counted.

Statistical analysis was performed separately for each no-choice test. The number of SA per test plant was subjected to analysis of variance using a generalized mixed model (PROC GLIMMIX, SAS Institute 2015; Littell et al. 2006) with Laplace likelihood approximation. The model used test line, sample day, and the test line × sample day interaction as factors, with test line and replicate as fixed factors and sample day as a random factor. The null hypothesis of equal aphid counts among soybean lines was tested using the Wald $F$ statistic (Quinn & Keough 2002). The LSMEANS procedure was used to separate mean aphid counts per plant per line for each sample day of the respective assays (Hesler 2013).

Results and Discussion

In all three tests, the number of SA per plant varied by test line, sample day, and the test line × sample day interaction (Table 2). Plants had more aphids per plant on Day 20 than on Day 10 across lines, and the significant interaction terms indicate that the magnitude of differences among test lines differed by sample day (Figures 1, 2, and 3).

On both sample days of the first test (Figure 1), the number of SA was lowest on LD05R-16137 (the resistant check), and the number on PI 437353 was lower than that of the remaining test lines. On Day 10, the susceptible lines Davison and SD01-76R did not differ in SA counts from one another or from PI 437658. By Day 20, the number of aphids on PI 437658 had become greater than on Davison, whereas counts on SD01-76R still did not differ significantly from those on Davison and PI 437658.

On Day 10 of the second test (Figure 2), the number of SA per plant was lowest on LD05R-16137, and the number on PI 437282 was lower than that on PI 437733. Counts of SA on PI 437733, PI 548417, Davison, SD01-76R and PI 548530 did not differ from one another, and counts on these lines did not differ from those
Fig. 1. Mean number of soybean aphids per plant on soybean lines in no-choice test 1 at 10 and 20 d after initial infestation. Bars denote ± SEM. Different lower case letters indicate significant differences in aphid numbers among lines on Day 10, and different uppercase letters denote significant differences in aphid numbers among lines on Day 20.

of PI 437282 and PI 437733. On Day 20, a few SA were still found on LD05R-16137. Numbers of SA on PI 548417, Davison, and SD01-76R did not differ from one another; numbers on these lines also did not differ from counts on PI 437282, but they were lower than counts on PI 437733 and PI 548530. Numbers of SA on PI 437282 did not differ from those on PI 437733 and PI 548530.

In the third test, numbers of SA per plant were lower on LD05R-16137 and PI 612759 C than the other four test lines on Day 10, and the other four lines did not differ from one another in the numbers of SA (Figure 3). On Day 20, the number of SA on PI 612759 C had become greater than that on LD05R-16137, but both of these lines still had fewer SA than the other four test lines. Numbers of SA on PI 548417 and Davison were lower than that on PI 512322 B. The number of SA on SD01-76R did not differ from the numbers on PI 548417, Davison and PI 512322 B on Day 20.

Two of the seven putatively resistant PI lines tested in this study had twice the level of resistance against an avirulent SA population than those on known susceptible soybean lines. However, these two lines, PI 437353 and PI 612759 C, also had SA levels that were greater than that on LD05R-16137, the resistant check. Thus, PI 437353 and PI 612759 C would be considered to have an intermediate level of SA resistance, but soybean breeders and pest management practitioners
have generally sought stronger sources of resistance in developing SA-resistant soybean cultivars (Hill et al. 2012, Hesler et al. 2013).

The development of durable aphid resistance in soybean cultivars is an important consideration in North America given that three virulent SA biotypes have been documented there against the major resistance genes *Rag1* (biotype 2; Kim et al. 2008), *Rag2* (biotype 3; Hill et al. 2010), and both *Rag1* and *Rag2* (biotype 4; Alt & Ryan-Mahmutagic 2013). Possible strategies of managing major resistance genes for durability in light of virulent biotypes include gene rotation, gene pyramiding, or planting isoline cultivars with different resistance genes in the same field (Smith 2005, Mundt 2014). With regard to gene pyramiding, some evidence suggests that incorporating minor resistance genes along with major resistance genes could boost resistance and theoretically enhance the durability of pyramided cultivars (Jun et al. 2013, Bhusal et al. 2017). Moreover, seeds of PI 437353 and PI 612759 C were respectively collected from locations in China and Russia (U.S. National Plant Germplasm System 2017), and thus they are expected to have different pedigrees and perhaps different genes contributing to SA resistance. As such, follow-up studies with PI 437353 and PI 612759 C may be useful to determine whether they might have valuable quantitative trait loci that could further diversify resistance genetics in soybean and contribute to a broader spectrum of resistance against SA biotypes (Wenger & Michel 2013, Mundt 2014).

Finally, despite the failure to find strong resistance in no-choice tests of the seven PI lines, follow-up studies are also warranted with the other sources of resistance identified in screening trials by Hesler et al. (2017a, b) in order to
increase the possibility of finding novel genes associated with SA resistance. Such efforts could potentially confirm new sources and thereby also broaden the spectrum of SA resistance, leading to prolonged utility of SA-resistant lines (Hodgson et al. 2012, Hesler et al. 2013, Hanson et al. 2016). The development of durable SA-resistant cultivars may provide an alternative to the heavy reliance on insecticides for control of this pest (Hodgson et al. 2012, Hesler et al. 2013).

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Harris, M. K. 1980. Arthropod-plant interactions related to agriculture, emphasizing host plant resistance, pp. 23–51. In M. K. Harris [Ed.], Biology and breeding for resistance to arthropods and pathogens in agricultural plants. Texas Agricultural Experiment Station, Texas A&M University, College Station, TX, and Agency for International Development, University of California, Berkeley, CA, 605 pp.


